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A Method for Concentrating Viruses Recovered from Sewage Sludges

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16. ABSTRACT

Buffered 10% beef extract eluates of primary, activated, and anaerobic mesophilically digested sludges were concentrated 20-fold by the Katzenelson organic flocculation procedure after diluting the beef extract in the eluates to a final concentration of 3%. The weighted mean recovery of virions from the concentrates was approximately 58% of the numbers present in the unconcentrated buffered 10% beef extract eluates. Flocculation of eluates that contained buffered 10% beef extract at times produced poor flocs. Application of the Katzenelson procedure to the diluted buffered 10% beef extract procedure for recovering virions from sludges permits an economy in the numbers of cell cultures required for assays of virions in beef extract eluates that is necessary in most laboratories.

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NOTE

A method for concentrating viruses recovered from sewage sludges

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Nous avons concentré par un facteur de 20 des éluats d'extrait de boeuf (10%) tamponné provenant des boues primaires, activées et digérées en anaérobiose sous des conditions mésophiles; nous avons utilisé pour ce faire la technique de floculation organique de Katzenelson après dilution de l'extrait de boeuf des éluats jusqu'à une concentration finale de 3%. Le taux de récupération moyen pondéré des virions à partir des concentrés était environ 58% des quantités présentes dans les éluats non concentrés de d'extrait de boeuf (10%) tamponné. La flocculation des éluats contenant l'extrait de boeuf (10%) tamponné donnait parfois de faibles précipités. En appliquant la technique de Katzenelson au procédé de récupération des virions de boues par l'extrait de boeuf (10%) tamponné, nous réalisons une économie dans le nombre de cultures cellulaires requises par la plupart des laboratoires pour les tests de virions dans les éluats d'extrait de boeuf.

[Traduit par le journal]

Introduction

Viruses indigenous to sludges may be bound to sludge solids or suspended in the liquid matrix. Viruses indigenous to sewage sludges can be recovered from those sludges by adsorbing suspended virions to the solids of the sludges and then eluting bound virions from the centrifuged sludge solids with buffered 10% beef extract (BE) (Berman et al. 1981). To reduce toxicity imparted from sludge extracts to cell cultures in which the virions are assayed, centrifuged sludge solids are suspended in a volume of BE elutant equal to that of the original sludge sample volume. Thus, the recovery procedure is not a concentration procedure. In this paper, we describe the concentration of virions into a relatively small volume of eluate with a modification of the Katzenelson organic flocculation procedure (Katzenelson et al. 1976).

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Material and methods

Cell cultures

Throughout these studies, virions were assayed in a continuous line of African green monkey kidney cells designated BGM (Dahling et al. 1974). The methods for growing and maintaining BGM cells in culture have been described in detail (Dahling and Safferman 1979).

The cells were propagated in 6-oz (1 oz = 28.4 mL) glass prescription bottles. The flat surface of the bottle on which the cells were propagated had an area of 45 cm^2. The confluent monolayers that formed were washed once with Earle's balanced salt solution just before they were inoculated.

Assays for virions

Virions were assayed by the plaque technic (Dahling et al. 1974). The cells used in this study were in passages 126–150. Drained cultures were inoculated with 1 mL of eluate, and the cultures were incubated at room temperature for 2 h. The inoculated cultures were then overlayed with a medium which has been described (Berman et al. 1981). Plaques were counted periodically and marked permanently when recorded.

Buffered 10% BE

Ten grams of beef extract powder (Lab-Lemco), 1.34 g of $Na_2HPO_4 \cdot 7H_2O$, and 0.12 g of citric acid were dissolved in

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100 mL of distilled water on a hot plate, and the resulting solution was autoclaved for 15 min at 121°C. The pH of the buffered 10% BE was 7.0 ± 0.1. The eluting capacity of each new lot of BE was compared with that of lots of known eluting capacity to determine that the capability of each new extract to elute at least several of the enteroviruses (poliovirus 1, coxsackievirus A9, echovirus 7) was maximal (Berg et al. 1971).

Membrane stacks

Membrane stacks consisted of 3.0-, 1.2-, 0.6-, and 0.45-μm Millipore MF membrane filters stacked together, in that order, in one filter holder. The filters had diameters of 47 mm. The filter stacks were used to remove bacteria and fungi from BE eluates.

Procedure

Primary, activated, and anaerobic mesophilically digested sludges were collected from sewage treatment facilities in southwest Ohio. Sludges were brought to the laboratory within 2 h after they were collected and hand-shaken until well mixed. Appropriate volumes of the sludges were dispensed into beakers and stored overnight at 4°C. The procedure for recovering and concentrating viruses from the sludges is shown in Fig. 1. $MgCl_2$ (final concentration = 0.05 *M*) or $AlCl_3$ (final concentration = 0.0005 *M*) was mixed vigorously into 100-mL volumes of the sludges, and sufficient 5 *N* HCl was added to each sludge sample to bring its pH to 3.0–3.5. The samples were mixed vigorously on magnetic stirrers for 30 min and centrifuged at 2500 × *g* for 15 min. The supernatants were discarded, the sediments were suspended in 100-mL quantities of 10% BE, and the suspensions were mixed on magnetic stirrers for 30 min to effect elution of virions. The suspensions were then centrifuged at 10 000 × *g* for 30 min. Each eluate (supernatant) was filtered through a membrane stack, and a portion of the filtered eluate was assayed. Another portion was diluted with distilled water to reduce the concentration of BE from 10 to 3% and the Katzenelson organic flocculation procedure (Katzenelson et al. 1976) was applied to the diluted BE. Thus, sufficient 5 *N* HCl was added to the diluted BE to bring the pH to 3.5, and the acidified BE was stirred for 30 min on a magnetic stirrer. The floc that formed was collected by centrifugation at 2500 × *g* for 10 min, dissolved in 10 mL of 0.15 *M* Na_2HPO_4, and assayed.

Results

Application of the Katzenelson organic flocculation procedure to buffered 10% BE eluates of the solids of primary sludges that had been salted with divalent (Mg^{2+}) or trivalent (Al^{3+}) cations and acidified with HCl to pH 3.0–3.5 at times produced poor flocs. Application of the same procedure to eluates first diluted to a BE concentration of 3% permitted a 20-fold concentration of the eluates and a weighted recovery of approximately 69% of the virions recoverable by direct inoculation of the BE eluates (Table 1A). Similar concentrations of eluates were achieved from activated sludges and anaerobic mesophilically digested sludges. The weighted virion recoveries from flocced BE eluates of activated sludges averaged approximately 58% of the numbers recovered by direct inoculation of eluates (Table 1B), and from flocced BE eluates of anaerobic mesophilically digested sludges averaged approximately 51% of the numbers recovered by direct inoculation of eluates (Table 1C). The recoveries of virions from the organically flocced eluates of sludges that had been salted with divalent cations and trivalent cations appeared to be about the same. The weighted mean overall recovery of virions from flocced BE eluates was approximately 58%.

Discussion

Viruses may be recovered from primary, activated, and anaerobic mesophilically digested sludges by treating the sludges with BE which elutes virions from the solids in the sludges. Since feces are the primary source of the viruses in sludges, the solids may be expected to contain a large portion of the viruses present in sludges. The efficiency of the recovery procedure may be increased by salting the sludges with divalent (Mg^{2+}) or trivalent (Al^{3+}) cations and acidifying the sludges to pH levels of 3.0–3.5 before eluting virions from the sludge solids with buffered BE (Berman et al. 1981). This procedure adsorbs the virions in suspension to the sludge solids and makes it necessary to recover virions only from the solids. Sludges, however, are often toxic to the cell cultures in which virions are recovered and assayed. Even the extracts of sludges that result when virions are eluted from sludge solids may be toxic. Thus, it has been necessary to elute adsorbed virions with large quantities of BE with the result that the recovery procedure has not been a concentration procedure also. Since the cell cultures used to recover and assay viruses are expensive, a procedure that reduces the volume of eluate that must be inoculated to detect and assay viruses is economically useful. The Katzenelson organic flocculation procedure (Katzenelson et al. 1976) serves this purpose and is thus an important asset to the technology. The range of recovery efficiencies for the study reported herein with the organic flocculation procedure is similar to that reported elsewhere (Sobsey et al. 1980).

The Katzenelson procedure did not work well with some lots of BE when the procedure was applied to eluates with a 10% concentration of BE. In such instances, the 10% concentration of the extract produced only small quantities of poorly formed floc. We resolved this problem by diluting the eluates so that they contained only 3% BE. The dilution, of course, increased the volumes of the eluates, but the quality and quantity of solids that precipitated when the pH of the eluates was reduced improved.

The Katzenelson procedure does not totally obviate the problem of toxicity to cell cultures. When flocced eluates were toxic, the toxicity was not reduced by washing the floc with acidified distilled water (pH 3.5) or by dissolving the floc in BE rather than in Na_2HPO_4.

Virus recovery procedure:
Sludge (100 mL)
Add $MgCl_2$ (to final concentration 0.05 *M*) or $AlCl_3$ (to final concentration 0.0005 *M*)
Salted sludge
On magnetic stirrer, adjust pH of salted sludge to 3.0–3.5 with 5 *N* HCl and mix vigorously for 30 min
Salted, pH-adjusted sludge
Centrifuge salted, pH-adjusted sludge at 2500 × *g* for 15 min
Discard supernatant; retain sludge solids
Sludge solids
Add 100 mL of buffered 10% BE (pH 7.0) to sludge solids
Mix resuspended sludge solids on magnetic stirrer for 30 min to elute viruses
Resuspended sludge solids
Centrifuge resuspended sludge solids at 10000 × *g* for 30 min
Discard solids; retain eluate (supernatant)
Eluate
Filter eluate through membrane stack to remove bacteria and fungi
Virus concentration procedure:
Filtered eluate (10% BE)
Assay filtered eluate (10% BE) for viruses
Add sufficient volume of distilled water to filtered eluate to reduce BE concentration from 10 to 3%
Begin Katzenelson organic flocculation procedure on filtered eluate (3% BE) as follows
Diluted filtered eluate (3% BE)
On magnetic stirrer, adjust pH of filtered eluate (3% BE) to 3.5 with 5 *N* HCl; *floc begins to form*
Mix filtered eluate (3% BE) and forming floc on magnetic stirrer for 30 min
Flocced eluate
Centrifuge flocced eluate at 2500 × *g* for 10 min
Discard supernatant; retain floc
Floc from eluate
Add 10 mL of 0.15 *M* Na_2HPO_4 to floc from eluate and mix
Floc dissolves on mixing
Dissolved floc
Assay dissolved floc for viruses

FIG. 1. Schematic representation of procedures for recovering and concentrating viruses in sludge.

TABLE 1. Concentration of virions from BE eluates of sludges

Treatment plant	Virions recovered from eluates (acidified $MgCl_2$-treated sludges), PFU*	Virions recovered from flocced eluates, %	Virions recovered from eluates (acidified $AlCl_3$-treated sludges), PFU*	Virions recovered from flocced eluates, %
		A. Primary sludge		
Sycamore	910	157	990	98
	107	37		
Mill Creek	312	41		
Lebanon	910	57		
Muddy Creek			2260	26
		Mean 73		Mean 62
		B. Activated sludge		
Sycamore	125	38	185	43
	160	73		
Muddy Creek	783	64	821	70
		Mean 58		Mean 56
		C. Anaerobic mesophilically digested sludge		
Mill Creek	345	50	377	41
	87	54	63	75
Lebanon	217	45		
Sycamore	80	41	76	41
Dayton	77	56	69	58
		Mean 49		Mean 54

*PFU, plaque-forming units.

Thus, it was at times necessary to dilute such eluates before they were inoculated onto cell cultures. Preliminary studies indicate, however, that treatment of floc with freon reduces toxicity, and this process will be studied further.

The Katzenelson procedure resulted in a reduction of virion recovery. It is not clear why this loss occurred. It is possible that some virions adsorbed to the glass of the vessels in which the procedure was undertaken or that the procedure itself was toxic to some virions. It is also possible that some interferences with virion adsorption to the floc occurred, that the efficiencies of virion recoveries varied from one strain of virus to another, or that substances concentrated from the sludge interfered with the adsorption of virions to the cell cultures. We plan to examine these possibilities.

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